Sporadic inclusion-body myositis: A degenerative muscle disease associated with aging, impaired muscle protein homeostasis and abnormal mitophagy

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1. Introduction

Sporadic inclusion-body myositis (s-IBM) is the most common degenerative muscle disease in which aging appears to be a key risk factor. In this review we focus on several cellular molecular mechanisms responsible for multiprotein aggregation and accumulations within s-IBM muscle fibers, and their possible consequences. Those include mechanisms leading to: a) accumulation in the form of aggregates within the muscle fibers, of several proteins, including amyloid-β42 and its oligomers, phosphorylated tau in the form of paired helical filaments, and we consider their putative detrimental influence; and b) protein misfolding and aggregation, including evidence of abnormal myoproteostasis, such as increased protein transcription, inadequate protein disposal, and abnormal posttranslational modifications of proteins. Pathogenic importance of our recently demonstrated abnormal mitophagy is also discussed. The intriguing phenotypic similarities between s-IBM muscle fibers and the brains of Alzheimer and Parkinson’s disease patients, the two most common neurodegenerative diseases associated with aging, are also discussed. This article is part of a Special Issue entitled: Neuromuscular Diseases: Pathology and Molecular Pathogenesis.

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Abstract

Sporadic inclusion-body myositis (s-IBM) is the most common degenerative muscle disease in which aging appears to be a key risk factor. In this review we focus on several cellular molecular mechanisms responsible for multiprotein aggregation and accumulations within s-IBM muscle fibers, and their possible consequences. Those include mechanisms leading to: a) accumulation in the form of aggregates within the muscle fibers, of several proteins, including amyloid-β42 and its oligomers, phosphorylated tau in the form of paired helical filaments, and we consider their putative detrimental influence; and b) protein misfolding and aggregation, including evidence of abnormal myoproteostasis, such as increased protein transcription, inadequate protein disposal, and abnormal posttranslational modifications of proteins. Pathogenic importance of our recently demonstrated abnormal mitophagy is also discussed. The intriguing phenotypic similarities between s-IBM muscle fibers and the brains of Alzheimer and Parkinson’s disease patients, the two most common neurodegenerative diseases associated with aging, are also discussed. This article is part of a Special Issue entitled: Neuromuscular Diseases: Pathology and Molecular Pathogenesis.

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1. Introduction

Sporadic inclusion-body myositis (s-IBM) is the most common degenerative muscle disease of older persons. The onset of clinical weakness is age-related, slowly progressive and leading to severe disability. There is no sustainable treatment available. Initially, s-IBM was considered a rare muscle disease, but during the last two decades, due to both physicians’ greater awareness of this disease and the existence of more reliable pathological markers of s-IBM muscle biopsies, diagnosis of s-IBM has become more prevalent.

The pathogenesis of sporadic inclusion-body myositis (s-IBM) is complex and involves multidimensional pathways, and several critical issues are still unresolved.

The known muscle-fiber degeneration aspects and mononuclear cell inflammation are components of the s-IBM pathology, but which is precedent and how they interrelate are not certain [1–5]. There is growing evidence that aging of the muscle-fiber, associated with intra-muscle-fiber accumulation of several conformationally-modified proteins, plays a primary and major pathogenic role leading to muscle-fiber destruction and clinical weakness.

s-IBM muscle-fiber degeneration is characterized by vacuolization and intra-muscle-fiber accumulation of misfolded, ubiquitinated, congophilic, multiple-protein aggregates [1–3]. We suggest that multiple posttranslationally-modified proteins (see below) accumulated within the s-IBM aging muscle fibers may be eliciting the T-cell inflammatory reaction. This may be similar to some older patients with hereditary IBM (h-IBM) caused by missense mutations in the UDP-N-acetylglucosamine-2 epimerase/N-acetylmannosamine-kinase (GNE) gene, whose muscle biopsies exhibit several similarities to s-IBM biopsies and have various degrees of lymphocytic inflammation [6–8], even though that form of h-IBM is considered not basically immune-mediated [9]. We propose that in older h-IBM patients, the “aging” muscle-fiber environment, and perhaps other individual intrinsic muscle fiber abnormalities, make some of the accumulated proteins interpreted as “foreign” by the immune system, thereby inducing the component of T-cell lymphocytic inflammation. Another hint that inflammation might be secondary in human s-IBM is provided by an alleged mouse model of s-IBM based on overexpression of mutated gelsolin D187N [10]. In that model, within the myofibers of aged mouse there were intra-myofiber accumulations of misfolded and...
congophilic proteins, including Aβ42 and gelsolin; in addition, there was perivascular and endomyosal lymphocytic infiltration, suggesting that inflammation in that mouse model was secondary to the overexpressed abnormal mutant protein or other proteins accumulated within the muscle-fiber. The possibility that in s-IBM the inflammation might be a secondary to the ongoing degeneration and production of abnormal proteins within the muscle-fibers could explain why s-IBM patients, in contrast to polymyositis patients, whose muscle biopsies express similar inflammatory repertoire, do not satisfactorily respond to various anti-dysimmune/anti-inflammatory treatments that have been extensively tried [4,11–13]. There is a possibility, not yet convincingly proven, that various cytokines and other putatively toxic products released from the inflammatory cells may contribute to s-IBM pathogenesis and aggravate the existing degenerative component — a concept proposed by others [14,15]. Another aspect involves a reported genetic predisposition to s-IBM involving the HLA-DRB1 genotype, as well as association of the HLA-A haplotype with earlier disease onset [16,17]. A recent paper reported that polymorphism in the TOMM40 gene influences the risk of developing s-IBM, as well as the age of disease onset [18]. TOMM40 encodes the mitochondrial pore protein Tom40, which is involved in the transport of Aβ42 and other proteins into mitochondria [19], and its elongation has been postulated associated with the pathogenesis of AD [20]. Even though still requiring confirmation, those studies might contribute to better understanding of mitochondri-al abnormalities in s-IBM, and add to the possible importance of Aβ42 in s-IBM pathogenesis.

An increase of non-organ-specific auto-antibodies, as well as association of some autoimmune disorders with approximately 27% of s-IBM patients was reported [16,21,22]. Auto-antibodies against S′-nucleotidase have also been reported in s-IBM patients [23,24], but their pathogenic significance is not yet known.

Here we describe several molecular abnormalities occurring in s-IBM musclefibers, which, in our opinion, importantly contribute to the s-IBM pathogenesis. These include: 1. Abnormal accumulation within s-IBM muscle fibers of multiprotein congophilic aggregates, which apparently result from a) increased transcription of several proteins, b) their abnormal posttranslational modifications and misfolding, and c) inadequate protein disposal. These phenomena indicate abnormalities of muscle protein homeostasis, also called “myoproteostasis” [25]. We propose that abnormal myoproteostasis might be provoked or aggravated by an aging intracellular milieu. 2. Consequences of impaired autophagy leading to accumulation of Aβ42 and its oligomers. 3. Abnormal-ITIES of mitochondrion putatively have a pathogenic role contributing to the mitochondrial abnormalities. Fig. 1 illustrates our current proposed cascade of s-IBM pathogenesis.

Experimental evidence indicating strong relationships among various pathologic pathways were recently described in detail [25], stressing the complex, interwoven pathogenic cascade of s-IBM.

Interesting phenomena involving degeneration of s-IBM muscle fibers are several similarities to the complex neuronal degenerations occurring both in Alzheimer (AD) and Parkinson (PD) brain diseases. These include: a) abnormal accumulations of many of the same putatively pathogenic proteins; b) similar posttranslational modifications of the accumulated proteins; c) similarly defective mechanisms of protein disposal, including inhibition of both the 26S proteasome and autophagy; and d) mitochondria abnormalities (detailed and referenced in [22,26,27]).

Accordingly s-IBM, similarly to AD and PD, is considered a “conformational disorder”, caused by protein unfolding/misfolding and associated with the formation of ubiquitinated multiprotein inclusion-bodies (aggregates) (reviewed and referenced in [2,5,26]).

**PROPOSED PATHOGENIC CASCADE OF s-IBM**

Fig. 1. Proposed pathogenic cascade of s-IBM. We propose that aging of the muscle fiber intracellular milieu, predisposing genes, and possibly some other aspects of the muscle-cell milieu, lead to still unknown molecular changes within the muscle fiber (indicated in the diagram as a black box with a question mark). This subsequently leads to several detrimental events, namely: a) existence of several abnormal mechanisms of protein transcription and processing; and b) accumulations of several proteins. These constitute the typical profile of s-IBM muscle fiber abnormalities (details in the text). We also propose that Aβ42 and its oligomers, as well as an inhibition of protein-disposal and mitochondrial abnormalities are key factors leading to muscle fibers atrophy, weakness, and death (details in the text).
Another important aspect of the s-IBM pathogenesis is an impaired regeneration ability of muscle fibers [28]. Whether a defective ability of s-IBM muscle fiber to regenerate partly relates to aging or other factors is not yet known [28,29].

Several reviews address clinical features of s-IBM, which are characterized by severe weakness of the quadriceps and forearm muscles, including severe weakness of finger flexors and extensors [4,12,13,21,30]. To diagnose s-IBM, there are no universally accepted serological tests. Even though antibodies against 5'-nucleotidase have been described by two groups and proposed to be a diagnostic marker for s-IBM [23,24], they are reported, depending on the study, in less than 50% of s-IBM patients. Furthermore, several non-s-IBM patients have positive 5'-nucleotidase antibodies [15]. Hence, the validity of those antibodies for s-IBM diagnosis awaits confirmation, and their pathogenic significance is not known.

Previously, increased serum amyloid-β 42 (Aβ42) was reported to be present in s-IBM, but not in polymyositis patients; however, that test has not received further consideration, probably because Aβ42 was also increased in a few older patients with previously, but possibly not correctly diagnosed “dermatomyositis” [31].

Currently, we consider that the most important diagnostic aspect of s-IBM is proper evaluation of the muscle biopsy.

2. Diagnostic criteria of the s-IBM muscle biopsy

Examples of the most typical abnormalities found in s-IBM muscle biopsies were recently described in details and illustrated [25–27]. Below, we provide a brief summary of the most characteristic diagnostic features.

2.1. Light-microscopy

2.1.1. Vacuolated muscle fibers

The characteristic feature of s-IBM on the Engel-trichrome staining is muscle fibers containing one or a few vacuoles in a given cross-section. Most of the vacuoles appear to be autophagic because they contain bluish-reddish material within the vacuoles or at their periphery, indicating lipoprotein membranous material.

2.1.2. Intracellular amyloid deposits

2.1.2.1. Congo-red staining. Multiple or single foci of β-pleated sheet amyloid, as identified by Congo-red fluorescence visualized through Texas-red filters, are evident within about 40–70% of the s-IBM vacuolated muscle fibers in a given transverse section, mostly in their non-vacuolated regions.

2.1.2.2. Crystal violet. Crystal violet metachromatic staining can also show the intra-myofiber amyloid deposits in s-IBM muscle fibers. While this method is more convenient because it does not require fluorescence microscopy, it is less precise because small amyloid deposits can be difficult to identify. Accordingly, we do not recommend this stain for routine evaluation of s-IBM muscle biopsies.

2.1.3. Intra-muscle fiber clusters of p62/SQSTM-immunopositive PHFs

p62/SQSTM1, or simply “p62”, is a shuttle protein transporting polyubiquitinated proteins for both proteasomal and lysosomal degradation (details below). In s-IBM muscle fibers p62 is an integral component of paired helical filaments (PHFs) that contain phosphorylated tau (p-tau). The staining appears in the form of strongly-immunoreactive, various-sized, mainly squiggly, linear or small rounded aggregates. These are usually in the vacuole-free cytoplasm of approximately 80% of the vacuolated muscle fibers, and in about 20–25% of the muscle fibers that appear “non-vacuolated” on a given 10 μm transverse section. Usefulness of p62 in immunocytochemical diagnosis of s-IBM was recently also emphasized by others [32].

2.1.4. Negative alkaline phosphatase (AP) staining of connective tissue

In s-IBM muscle biopsies, perimysial connective tissue, even in regions of active disease, lacks the typically-strong AP-positivity seen in similarly-active regions of polymyositis (PM) and dermatomyositis (DM) biopsies — we attribute this activity to active fibroblasts [reviewed in [4]]. It is a convenient and inexpensive stain to differentiate between s-IBM and PM [4].

2.1.5. Staining with novel fluorescent amyloid ligands

Fluorescent amyloid ligands termed luminescent conjugated oligothiophenes (LCOs), rapidly and with high sensitivity detect protein inclusions in s-IBM muscle fibers [33]. Their use requires fluorescence microscopy, and they are not yet commercially available, to our knowledge. However, the easiness of their use and high sensitivity should make them very attractive in the future.

2.1.6. Typical but not diagnostic abnormalities

2.1.6.1. COX-negative fibers and ragged-red fibers. Characteristic, but not diagnostic of s-IBM, are cytochrome oxidase (COX)-negative muscle fibers and ragged-red fibers — both those abnormalities are occurring more often in s-IBM as compared to aged-matched normal controls [34].

2.1.6.2. TDP-43 immunoreactivity. Transactive response DNA-binding protein 43 (TDP-43) is immunoreactive within s-IBM muscle fibers in the form of cytoplasmic inclusions, and reported to be diagnostic, even though they were not present in a significant number s-IBM muscle biopsies [35,36]. However, fibers positive for p62-inclusions were reported three-times more frequent than ones containing TDP-43-inclusions [37]. Moreover, p62-inclusions were more sharply defined, and two biopsies displaying several p62 inclusions did not contain any TDP43-inclusions. Therefore, we do not recommend TDP-43 for diagnostic evaluation of s-IBM biopsies.

2.2. Electron-microscopy

Previously, electron-microscopic (EM) identification of PHFs was considered important for s-IBM diagnosis. Now, because of the availability of new histochemical and immunohistochemical markers, that importance has diminished. For example, following staining with the antibodies against p62 or p-tau, typical clusters of PHFs are easily visualized by light microscopy — these we have demonstrated by EM-immunocytochemistry strongly associated with PHFs.

3. General pathogenic considerations

In our opinion, it is becoming more evident that in s-IBM a unique type of muscle-fiber degeneration leads to muscle-fiber atrophy, and also resulting in muscle fiber degeneration and death — these are manifesting clinically by inexorably progressive muscle weakness.

Presumably, the demonstrated 265 proteasome and autophagy inhibitors contribute to the observed abnormal protein aggregates, β-pleated sheet amyloid accumulation, and cytoplasmic vacuolization.

Abnormal accumulation of ubiquitinated intracellular proteinacious congophilic inclusions is characteristic of the s-IBM phenotype — accordingly, s-IBM, similarly to AD and PD, is considered a “conformational disorder” caused by protein unfolding/misfolding and associated with the formation of multiprotein inclusion-bodies (reviewed in [1,3,5]). As with AD and PD brains, in s-IBM muscle fibers the sequence of detrimental pathologic events comprising cellular degeneration is not well delineated. Even though it is not clear whether protein-aggregates themselves impair normal cellular functions, it is very likely that they are detrimental to the muscle fiber, because masses of aggregates — which are visible in many muscle fibers on a given transverse section and known to be present in various places along the muscle fibers — could severely...
impair muscle fiber integrity and mechanical function. Large aggregates can physically displace and disturb the function of normal cytoplasmic proteins and organelles, such as mitochondria and endoplasmic reticulum. Proteins that accumulate within the aggregates often show oxidative damage [38], which may have been caused them to be incorporated there. Oxidative and nitrosytrosine stress occurs in s-IBM muscle fibers [39,40]—and both affect assembly and phosphorylation of tau [41,42], and probably damage various other proteins, thereby impairing muscle-fiber function.

Below we describe the most recent research directed toward understanding the mechanisms causing impaired protein degradation, protein molecular modifications, abnormal protein aggregation, and related consequences. Also discussed are our newest findings related to abnormal mitophagy. The putative pathogenic importance of the endoplasmic reticulum stress that we have identified in s-IBM muscle has been previously discussed [25,26].

4. Abnormal myoproteostasis

In normal cells, quality-control mechanisms assure that any damaged or malfunctioning intracellular structures, including proteins and organelles, are identified and repaired, or removed (reviewed in [43, 44]). The cellular mechanisms for ensuring proper protein “quality-control” also influence protein transcription, thereby preventing proteins from being over- or under-produced. To eliminate misfolded proteins, a cell utilizes: a) protein refolding through the endoplasmic reticulum (ER)-chaperones; b) protein refolding through heat shock proteins; c) protein degradation through the 26S ubiquitin-proteasome system (UPS); and d) protein degradation through autophagy. Under various pathological conditions, and in aging, protein quality-control is disturbed (reviewed in [43,45]). This results in accumulation and aggregation of pathologically modified proteins and damaged organelles.

The term “proteostasis” describes the integrated cellular network that controls the life of proteins from their initiation to termination [46,47]. Proteostasis is considered a broader term than “protein quality-control” because it encompasses regulation of protein transcription, translation, folding, processing, assembly/disassembly and degradation [46,47]. Although some abnormal aspects of proteostasis are present in every abnormal cell, abnormal proteostasis is considered especially important in several neurodegenerative diseases of brain [46] and in s-IBM muscle fibers, where accumulated protein aggregates contain various proteins in different stages of abnormal proteostasis [25].

Among several proteins (details reviewed in [26]), we here focus only on a few likely to be pathogenically most important in s-IBM. Conceptually, proteins identified as increased or immunocytochemically-present in an aggregate could be the result of their i) overproduction, ii) impaired catabolism (related to proteasome or lysosome inadequacy), or iii) post-translational modification, including being “attached” to other accumulated proteins. Accumulated proteins a) might have abnormal structure causing their aggregation, or b) might have their normal cellular structure, and/or function, but are “captured” into an aggregate of an abnormal protein. Collectively, we apply to the term “abnormal myoproteostasis”.

4.1. Increased transcription and accumulation of amyloid-β precursor protein (Aβ/PP), and abnormalities of Aβ/PP processing, involving BACE1 and γ-secretase complex

S-IBM muscle fibers have increased mRNA of Aβ/PP-751 [48], the most abundant form in the peripheral tissues [49]. The underlying mechanism of the Aβ/PP overproduction in s-IBM is not yet clarified. In addition, Aβ/PP in s-IBM muscle fibers is posttranslationally modified, as evidenced by its increased phosphorylation [50]. Importantly, there are also distinct abnormalities in Aβ/PP processing, including increased transcription and accumulation of BACE1 ([γ-site amyloid-β precursor protein cleaving enzyme] [26,51], which cleaves Aβ/PP at the N-terminal of Aβ [52], and increased levels of BACE1-antisense (BACE1-AS) transcript [51], a non-coding RNA that regulates BACE1 mRNA and protein expression in vivo and in vitro, and was reportedly also increased in AD brains [53]. It would be of interest to study whether other non-coding RNAs are accumulated in s-IBM muscle fibers. The importance of non-coding RNAs in various pathological processes was recently reviewed [54].

Also demonstrated were increased transcription and accumulation of the components of the γ-secretase system [55], which cleaves Aβ/PP at the C-terminal of Aβ to generate either Aβ40 or Aβ42 [reviewed in (56)]. Additionally, Nicastrin, a component of the γ-secretase complex, is strongly hyperglycosylated in s-IBM, indicating its posttranslational modification [55]. Other factors likely contributing to Aβ production, deposition and oligomerization, such as cystatin C, transglutaminases 1 and 2, and cholestero are also increased in s-IBM muscle fibers (details in [3,26]). Accordingly, transcription of Aβ/PP combined with the milieu within the s-IBM muscle fiber is a facilitating environment for Aβ production and accumulation.

4.2. Accumulation of Aβ42 and evidence of putatively toxic Aβ42 oligomers

Our studies of s-IBM muscle biopsies more than two decades ago were the first to identify an intracellular accumulation of Aβ in any disease [57,58]. They were the basis for our proposal of an important cytotoxic role of intra-cellular Aβ, not only for s-IBM muscle fibers but, by analogy, suggested also for AD neurons [59]. Previously for years it had been considered that only extracellular Aβ is detrimental in AD brain [60]. Now, however, the presence of intraneuronal Aβ is well established and its possible toxicity and importance in the AD pathogenesis are being considered (reviewed in [46,61,62]).

In s-IBM muscle fibers, Aβ42 is the form associated with the Congo-red-positive amyloid inclusions [63]. Recently, we have shown that dysferlin is a new binding partner of Aβ42 [64]. This is of particular interest, because in s-IBM dysferlin was absent or greatly decreased from its normal location at the periphery of the muscle fibers, but was accumulated, together with Aβ42, within the muscle fibers in the form of large aggregates [64]. Since genetically-dysferlin-deficient patients often have inflammation in their muscle biopsies [65], one can wonder whether a) dysferlin absence from the periphery of s-IBM muscle fibers might contribute to s-IBM inflammatory reaction, or b) perhaps the pre-aggregated dysferlin protein molecule is subtly altered so as to make it “foreign” to the patient’s immune system.

The putative importance of Aβ42 cytotoxicity in s-IBM muscle fibers is strengthened by our demonstration of actual Aβ42 oligomers in them [66]. In general, cytotoxicity of Aβ is considered to depend on its initial assembly into detrimental oligomers. In contrast to Aβ monomers, which in other systems are considered not cytotoxic (recently reviewed in [61,62]), small oligomers and protofibrils are thought to be the cytotoxic forms of Aβ42 [46,61,62,67]. In s-IBM muscle biopsies, Aβ42 dimers, -trimers and -tetramers were evident by immunobots [66], thereby providing additional evidence that intra-muscle-fiber accumulation of Aβ42 oligomers may contribute importantly to the s-IBM pathological cascade. Nonfibrillar, cytotoxic “Aβ-Derived Diffusible Ligands” (ADDLs), originally derived from Aβ42 [67], were shown increased in AD brain and proposed to play an important pathogenic role [67], and were also increased in s-IBM muscle [66]. (Actually, the principle of a cytotoxicity of soluble, ultrastructurally-invisible pre-fibrillar abnormal amyloidogenic proteins was postulated 3 decades ago for extracellular amyloidoses [68]).

4.3. Phosphorylated tau

In s-IBM muscle fibers, as in AD brain [69,70], p-tau is accumulated intracellularly in the form of congophilic, squiggly or linear inclusions [3,25,26,71], which is identified by various antibodies [71,72], recognizing several of the same epitopes of p-tau that are present in AD brain, including ones specifically identifying “AD-specific” conformational tau
[25,73,74]. By immuno-EM, p-tau appears as paired helical filaments (PHFs). Several kinases known to phosphorylate tau [69,70] are also accumulated within s-IBM muscle fibers, where they co-localize with p-tau-positive inclusions. These include extracellular signal-regulated kinase (ERK), CDK5 (reviewed in [26]), glycogen synthase kinase 3β (GSK-3β), and casein kinase 1 [75]. Also, GSK-3β is hyperphosphorylated and activated in s-IBM muscle fibers [50].

s-IBM-PHFs also contain RNA and the RNA-binding-protein Survival Motor Neuron (SMN), both of which were proposed to contribute to PHF formation [76]. Studies related to neurodegeneration strongly suggest that intracellular increase of p-tau is cytotoxic to neurons (reviewed in [70,77]). Recently, it has been reported that in AD brain p-tau is hyperacetylated due to deficiency of SIRT1 [78]. That modification was reported to impair p-tau degradation by the 26S proteasome [78]. Impaired autophagy has also been implicated as a factor contributing to tau oligomerization and accumulation [79]. It is possible that Aβ42-oligomers, impaired functions of both the 26S proteasome and autophagy, and decreased SIRT1 activity, all identified in s-IBM muscle fibers [63,66,80,81], contribute to abnormal tau phosphorylation in s-IBM. It has also been proposed that Aβ42 can trigger conversion of tau into a toxic state and, in turn, toxic tau enhances Aβ toxicity [82]. As both proteins co-exist in s-IBM muscle fibers, it is quite possible that their pathologic interaction occurs in s-IBM.

Several other proteins are also accumulated within the bundles of p-tau-containing PHFs (referred in [26]), and it is possible that multiple clusters of PHFs might significantly displace and impair the function of other cytoplasmic proteins and organelles, such as mitochondria and endoplasmic reticulum. Also, pre-aggregated abnormal misfolded tau molecule might have an invisible, submicroscopic “sticky” cytotoxicity (see above).

4.4. Myostatin (MSTN)

MSTN, a protein secreted from skeletal muscle, is considered a negative regulator of muscle growth during development and of muscle mass during adulthood [83]. In biopsied s-IBM muscle fibers, MSTN precursor protein (MSTNPP) and MSTN dimers are significantly increased on immunoblots, and MSTNPP immuno-co-localizes with Aβ/APP [84]. Interestingly, APP-overexpression into cultured normal human muscle fibers increased MSTNPP expression, and subsequent experimental inhibition of proteasome caused accumulation and colocalization of both MSTNPP/myostatin and Aβ/APP/Aβ, and their physical association [85]. The mechanism(s) by which overexpressed Aβ/APP/Aβ increases MSTNPP is not known. Possibly, Aβ/APP binding to MSTNPP causes its posttranslational modification that lessens its traffic and degradation, resulting in accumulation – it is unknown whether in that hypothetical situation myostatin would retain its deleterious effect on muscle fibers.

Recently, the importance of MSTNPP accumulation in s-IBM was emphasized by the studies of others [86] demonstrating that MSTNPP is capable of forming intracellular β-pleated sheet amyloid. Since MSTN physically associates with Aβ/APP [84,85], it is possible, as we have previously proposed [84], that these two proteins might enhance each other’s aggregation, oligomerization and β-pleated sheet formation.

4.5. α-Synuclein

α-Synuclein (α-syn) is a protein of poorly understood normal cellular function. It is a major component of Lewy bodies (LB) in PD brain (reviewed in [87,88]).

Abnormal expression of α-syn occurring spontaneously in the brains of various neurodegenerative disorders, including AD brain, has been associated with, and possibly causative of, oxidative stress, impaired proteasome function, and mitochondrial abnormalities [87]. Oxidative stress can induce aggregation of α-syn into amyloid-like fibrils [87]. In s-IBM muscle α-syn is also increased, by immunohistochemistry and immunoblots [89,90], and its mRNA is increased by real-time PCR, suggesting its increased production (Nogalska et al., 2011, unpublished). In s-IBM muscle, the increased α-syn might also be due to its decreased degradation. α-syn is degraded by both 26S proteasome and autophagy (recently reviewed in [91]), both of which are impaired in s-IBM muscle fibers [80,81]. Moreover, abnormalities of chaperone mediated-autophagy (CMA), an important pathway of α-syn degradation, have recently been reported in s-IBM muscle fibers [92].

α-syn has been shown to be associated with mitochondria, and its overexpression induced mitochondria abnormalities (reviewed in [93]). Because oxidative- and nitric-oxide-induced stresses, and mitochondrial abnormalities, are also aspects of the s-IBM muscle-fiber pathology [139,40], and reviewed in [22,26] and below), a putative toxicity of α-syn may contribute to the muscle-fiber degeneration. Recently, α-syn was reported to directly stimulate tau phosphorylation through activation of GSK-3β [94].

5. Abnormal protein disposal

Unfolding or misfolding of proteins can occur in vivo and in vitro under several circumstances, including macromolecular crowding, defective protein disposal, oxidative stress, and “aging”. Intracellular mechanisms intended to maintain a proper quality and balance of proteins and organelles try to assure that any malfunctioning or damaged intracellular structures, including proteins and organelles, are identified and repaired or cleared (reviewed and referenced in [26,43,91]). This control or surveillance machinery is particularly important for the non-dividing post-mitotic cells like neurons or muscle fibers, because their accumulated abnormal proteins cannot be diluted during cell division [91]. Under various pathological conditions and in aging, protein quality-control is disturbed (reviewed in [46,91,95]).

In eukaryotic cells, two major pathways of cellular protein degradation relate to the 26S proteasome and the autophagic/lysosomal systems [43,91,95]. The 26S proteasome, also called the ubiquitin-proteasome system (UPS), is a major degradation mechanism for: a) normal regulatory and other short-lived proteins, and b) misfolded proteins exported from the endoplasmic reticulum (ER) through a ubiquitin-mediated ATP-independent process [43]. In contrast, long-lived, structural proteins and/or various damaged or misfolded proteins, abnormal mitochondria and other obsolescent cellular organelles are degraded through “autophagy” [91,95].

5.1. Impairment of the 26S proteasome function in s-IBM muscle fibers

In s-IBM muscle fibers, our studies demonstrated proteasome impairment as evidenced by reduced activities of the three major proteasomal proteolytic enzymes [80]. Our experimental studies suggested that Aβ/APP/Aβ may inhibit proteasome function [80]. Other factors present in s-IBM muscle fibers that might contribute to inhibiting proteasome function include: an aging muscle-fiber environment; protein over-crowding; oxidative stress; and accumulated p-tau, α-synuclein, and UBB+1 [96] – all of these are capable of inhibiting proteasome activity in other systems (referenced in [26]).

A failure to degrade/remove unnecessary proteins, including various abnormal damaged proteins, is presumably as detrimental to muscle fibers as it is to other cells. For example, accumulated ubiquitinated, misfolded, and oxidized proteins aggregate by themselves and can cause proteasome inhibition, and might do so in their pre-aggregated phase. It is more likely that the still-soluble, early intermediates of protein aggregates, in the form of dimers and trimers, can also induce proteasome inhibition, and some are known to be highly toxic to cells. There are other diverse functions controlled by the ubiquitin-proteasome system (UPS), including regulation of gene transcription through monoubiquitination and deubiquitination of histones, and presentation of major histocompatibility complex I (MHCI) (reviewed in [97]). Whether proteasomal
abnormalities relate to antigen presentation and T-cell inflammation in s-IBM muscle fibers is not known.

5.2. Impaired autophagy in s-IBM muscle fibers

Autophagic pathways are composed of three main components 1) macroautophagy, 2) chaperone-mediated-autophagy (CMA), and 3) microautophagy, all of which lead to cargo degradation within the lysosomes (reviewed in [91,98,99]). Lysosomes comprise the main compartment in which degradation of various proteins and other molecules actually occurs, through the activity of various lysosomal enzymes. When the lysosomal function is inhibited, there is proliferation and enlargement of autophagosomes “cargo-shippers”, which constitute the route by which the cargo is transferred to the lysosomes, because the cargo that they are carrying cannot be received and cleared by the lysosomes. That situation is detrimental to the cell and can result in formation of autophagosomal vacuoles [95]. This occurs in s-IBM muscle fibers, as well as in neurons of some neurodegenerative disorders (reviewed in [3,26,91,95]). Although autophagic vacuoles associated with accumulated lysosomal-membranous structures within s-IBM muscle fibers have been associated with s-IBM pathology for many years, the mechanism of their formation was not well understood. Our recent studies have demonstrated that in s-IBM muscle fibers there is increased formation and “matura
tion” of pathologic vacuolar autophagosomes [81]. These observations suggested that excessive activated macroautophagy is an important factor leading to formation of the vacuoles.

In addition to activated macroautophagy in s-IBM muscle fibers, our recent studies provided important evidence that autophagy related to lysosomal function is diminished [81]. Those changes appeared to be specific to s-IBM, because we found in polymyositis muscle fibers that lysosomal activities were actually increased [81], thereby suggesting that the seemingly similar lymphocytic inflammation present in both s-IBM and PM might not contribute to impairment of the autophagic/lysosomal degradation in s-IBM.

Impaired autophagy in s-IBM muscle fibers could be, at least partially, responsible for some of the abnormal accumulation of various proteins, including Aβ, α-syn, BACE1 and tau, all reported to be degraded through autophagy [52,91,100,101]. Of uncertain pathogenic significance, Aβ has been found to be produced within the autophagosomes [95,102].

5.3. Abnormalities of p62 and NBR1

Two partner proteins, p62 and NBR1 (neighbor of BRCA1 gene 1), which are both shuttle proteins transporting polyubiquitinated proteins to either proteasomal or lysosomal degradation [103–105], are greatly increased in s-IBM muscle fibers [72,106], where they accumulate in the p-tau-containing PHFs structures. In AD neurofibri
tillary tangles (NFTs) and Lewy bodies of Parkinson-disease, p62 was found accumulated in those ubiquitinated inclusions [107]. In AD brain, p62 localized in NFTs was associated with phosphorylated tau (p-tau) [107]. Our recent studies similarly demonstrated that in s-IBM muscle both p62 and NBR1 are increased at both the protein and mRNA levels, and are strongly accumulated in aggregates within muscle fibers, where they closely colocalize with p-tau by both the light- and electron-microscopic immunocytochemistry [72,106]. This is another example of impaired protein degradation in s-IBM muscle fibers. It is of interest that it was recently reported that a) NBR1 is phosphorylated by GSK-3β, b) proper phosphorylation of NBR1 prevents its aggregation, and c) in s-IBM NBR1 is hypo-phosphorylated [108]. The study is of interest because some other proteins present in s-IBM appear hyperphosphorylated by GSK-3β [50,109], which might suggest that some type of modification of NBR1 prevents its proper phosphorylation.

5.4. Mitochondrial abnormalities, including abnormal mitophagy

Mitochondrial abnormalities, including ragged-red fibers [110], cytochrome-oxidase (COX) negative muscle fibers (at a given transverse level), and multiple mitochondrial DNA deletions, are more common in s-IBM muscle than expected for the patient’s age (reviewed in [34]). Other common abnormalities in the s-IBM muscle biopsy are: a) very enlarged “giant” mitochondria, many of which have paucity of cristae (Askanas et al, unpublished observation), and b) many mito
chondria having paracrystalline inclusions. An antioxidant DJ-1 [111] is an important mitochondrial protective agent (referenced in [39]). DJ-1 was demonstrated quantitatively increased in s-IBM muscle fibers, where it is highly oxidized and abnormally accumulated in mitochondria [39], suggesting that the increased DJ-1 may be attempting to mitigate mitochondrial oxidative damage, but may be ineffective because it is itself excessively oxidized.

Downregulated expression of complex I of the mitochondrial respira
tory chain, and mitochondrial DNA rearrangements in the majority of individual respiratory-deficient muscle fibers have recently been reported in s-IBM [112]. Those authors reported a correlation between the number of T-lymphocytes and macrophages residing in muscle tissue and the abundance of respiratory-deficient muscle fibers [112]. We have not observed any correlation between COX-negative muscle fibers and mononuclear inflammation in s-IBM muscle fibers [113].

In general, the mechanisms causing mitochondrial abnormalities in s-IBM are not clarified, but possibilities include: a) toxic soluble oligo
mers of Aβ/42, α-syn, p-tau, or other proteins; b) factors related to the demonstrated oxidative and ER stresses; and c) abnormal autophagy. Our newest studies point to the abnormalities of mitophagy, a process involving degradation of mitochondria through the lysosomal system [114–117]. Abnormal mitophagy has also been linked to aging and various neurodegenerative disorders (reviewed in [116,118]).

Cellular elimination of dysfunctional mitochondria through mitophagy involves sequestration of mitochondria into LC3-II-containing autophagosomes, which subsequently deliver them to lysosomes for degradation [116–118]. The mitophagy receptor Bcl-2 adenosine Virus E1B19 19-kDa interacting protein (Bnip3) through its homodimerization and subsequent binding of its dimer to LC3 en
dables recruitment of damaged mitochondria to the autophagosomes [119].

Mitochondrial dynamics importantly participate in the regulation of mitophagy; increased mitochondrial fission is necessary for mitochondrial degradation, but enhanced mitochondria fusion results in mitochondrial enlargement that obstructs their clearance [117,120,121]. We have recently a) immunolocalized Bnip3 and Mfn1, a marker of mitochon
dria, in s-IBM muscle fibers and control muscle fibers, and b) studied Bnip3 and Mfn1, in mitochondria isolated from s-IBM and age-matched normal-control muscle biopsies, and c) analyzed Bnip3 and Mfn1 mRNAs in total muscle homogenates by real-time PCR [122].

Our studies showed that in s-IBM as compared to controls, there was a) a significant increase of both Bnip3-dimer and of Bnip3 mRNA, and b) a significant increase of Mfn1, its mRNA [122] and Nogalska et al. in preparation. Interestingly, in s-IBM, Bnip3 was accumulated in the form of aggregates within muscle-fibers, where it closely co-localized with LC3 (Fig. 2).

By immuno-electron-microscopy, Bnip3 was mainly located on the outer mitochondrial membrane (Fig. 3) and it closely co-localized with LC3 within very abnormal mitochondria (Fig. 3).

(We were not able to immunohistochemically detect Bnip3 in either normal or diseased control muscle biopsies [in those biopsies, LC3 has been shown negative by us previously [81]].) Interestingly, Bnip3 and Mfn1 were strongly immunoreactive at the periphery of ragged-red fibers, which was not s-IBM-specific (Fig. 2). We previously demonstrated that in various diseased human muscle, α-syn and parkin accumulate in ragged-red fibers (reviewed and illustrated in [26]). We propose that ab
normal mitochondria within ragged-red fibers, in various diseases
FIG. 2. Light-microscopic immunolocalization of Bnip3 and Mfn1. Upper row. Immunolocalization of Bnip3 and Mfn1 in ragged-red fibers. Immunostainings and Engel-trichrome staining were performed on serial transverse sections. Ragged-red fibers are identified as having a red staining at the periphery of the fiber, which is associated with the collection of very enlarged abnormal mitochondria [107]. Increased staining of both Bnip3 and Mfn1 occurs within abnormal mitochondria of the ragged-red fibers. All × 900. Middle row. Single and double-immuno-labeling of Bnip3 indicates its strong accumulation in the form of various-sized aggregates in s-IBM muscle fibers. In a double-labeled example, Bnip3 closely co-localizes with LC3, suggesting its presence within the autophagosomes. All × 1400. Lower row. Single and double-immuno-labeling of Mfn1 in s-IBM muscle fibers indicates its strong accumulation in the form of various-sized aggregates. In a double-labeled example, aggregates of Mfn1 closely co-localize with Bnip3. All × 2500.

FIG. 3. Single and double gold-immuno-electron-microscopy of Bnip3 and Mfn1 in s-IBM mitochondria. Gold particles indicate that Bnip3 is localized at the periphery of the mitochondrion in the form of small clusters; in one very abnormal mitochondrion, probably designed for autophagosomal degradation, Bnip3 is intermingled with LC3 (Bnip3 6 nm gold particles, LC3 12 nm gold particles). Mfn1 is increased at the site where the mitochondria are presumably fusing, as suggested by concentration of 5 nm gold particles at those sites. All 82,000.
including s-IBM, are destined for autophagic degradation, and parkin is recruited to facilitate their clearance, as has been reported in other systems (reviewed in [118]).

In s-IBM muscle the increase of mitophagy receptor Bnip3 and its binding with LC3 suggest that the signal for mitochondria transport to the autophagosomes is preserved and even increased. However, in s-IBM muscle the mitochondrial degradation is likely impaired due to i) enlargement of the mitochondria resulting from their increased fusion; ii) accumulation of Bnip3 in the form of aggregates, which might inhibit its activity; but mainly due to iii) our previously-demonstrated lysosomal inhibition, all of which might lead to the accumulation of damaged mitochondria. It has been demonstrated recently that experimental overexpression of UBB +1 increases mitochondrial fusion proteins, but it decreases mitochondrial fission proteins [123]. UBB +1 is accumulated in s-IBM muscle fibers [96], but whether or not UBB +1 increase has an influence on mitochondrial fusion in s-IBM is not currently known.

The mitochondrial abnormalities in s-IBM muscle seem likely to contribute to the muscle-fiber malfunction, degeneration, and weakness. The importance of the mitochondrial unfolded protein response (UPRmit), and the involvement of the mitochondrial unfolded protein response (UPRmit) have recently been emphasized [47,124].

6.1. Sodium phenylbutyrate (NaPB)

NaPB is an orally bioavailable small molecule approved by the FDA for treatment of urea cycle-disorders [125,126]. Taken daily and long-term by infants, children and adults, it is usually well-tolerated [126]. NaPB reportedly mimics the function of intracellular molecular chaperones by preventing protein aggregation and oligomerization [127], in addition to being a histone deacetylase inhibitor [124,128].

In our experimental human muscle tissue culture IBM model based on inhibited autophagy (Autoph (-/-)-IBM-HM-TC-Model), treatment with NaPB: a) virtually completely eliminated vacuolization; b) increased activities of both cathepsins D and B; c) decreased NBR1 and p62; d) substantially decreased Aβ42 and Aβ42 oligomerization; and e) decreased γ-secretase activity [128]. All those parameters are substantially increased both in s-IBM muscle fibers and in our Autoph (-/-)-IBM-HM-TC-Model [81,128]. Unblocking a defective lysosomal degradation as a possible therapeutic strategy for s-IBM and neurodegenerative diseases was previously proposed [25,129], and experimental restoration of cathepsin B, D, and L activity in transgenic AD mice resulted in Aβ clearance and improved AD-like pathology in them [129]. Our results show that NaPB improves lysosomal activity and ameliorates the consequences of the impaired autophagy, providing a rationale for considering NaPB as a putative drug for s-IBM patients, and possibly other neurodegenerative disorders with impaired lysosomal degradation.

These data provide a strong rationale for considering therapeutic trials of s-IBM patients with NaPB.

6.2. Lithium

Lithium was reported to diminish tau and Aβ42 pathologies in various experimental models of AD (reviewed in [130]). In a transgenic mouse model whose skeletal muscle bears some aspects of IBM muscle fibers, lithium was reported to decrease tau phosphorylation through decreasing activity of GSK-3β [131]. In cultured human muscle fibers in our Aβ42-overexpressing IBM model (Aβ42(+)-IBM-HM-TC-Model), lithium significantly decreased total Aβ42, phosphorylated Aβ42, and Aβ42 oligomers [50]. In addition, lithium significantly increased the inactive form of GSK-3β [50]. Accordingly, treating s-IBM patients with lithium, which is widely used in treating bipolar disorders in humans, might be beneficial if used in “adequate” and safe dosage.

6.3. Polyphenols

Polyphenols recently have been reported to benefit experimental mouse models of AD and an IBM–culture model. For example: a) treatment with Resveratrol (trans-3,4′,5-trihydroxystilbene), an antioxidant polyphenol and potent activator of SIRT1 (reviewed in [132]), in our ER stress-induced cultured human muscle fibers (ERS+/-IBM-HM-TC-Model) significantly: i) decreased myostatin mRNA and protein, which was associated with NF-κB de-acetylation (de-activation), and ii) increased muscle-fiber size [133], suggesting that resveratrol might be beneficial in treating s-IBM patients if one can use a stable non-toxic form in adequate dosage. b) Other phenolic compounds, including curcumin and grape-seed-derived polyphenols have been reported to decrease the amyloid-burden and Aβ fibrillation in AD transgenic mouse models and in vitro [134].

In the future other treatment approaches might include a) mitochondria protective molecules, b) decreasing myostatin, and c) decreasing the consequences of the ER stress.

Several approaches, including active and passive immunization against Aβ42 have been proposed for treatment of AD but, despite their possible effects in various AD mouse models, their usefulness for patients is still unknown. Some of the trials had to be interrupted, either because of detrimental side-effects or lack of the evident efficacy. Those studies were recently in [135,136]. Other approaches, which are also being discussed as possible treatments of AD, are a) BACE-1 inhibitors, and b) tau-immunotherapy, reviewed in [136,137]. However, their application to human patients still remains unknown. If any of those approaches will prove safe and beneficial for AD patients, that would justify treatment trials in s-IBM patients.

However, their possible toxicity and potential benefits for s-IBM patients are still not known.

7. Conclusion

In conclusion, s-IBM is a degenerative muscle disease in which aging appears to be a key risk factor. Several molecular mechanisms responsible for multiprotein aggregation and accumulation within s-IBM muscle fibers were reviewed. Since anti-dysimmune treatments are generally not effective, and were even reported to be detrimental for s-IBM patients [12], it is not likely that s-IBM is a primary dysimmune/inflammatory disease. But as we have been proposing, accumulation of
posttranslationally modified, misfolded proteins in the aging milieu of s-IBM muscle fibers might be perceived by the patient’s immune system as “foreign” (not “self”) and be responsible for inducing T-cell inflammation in the s-IBM muscle. Accordingly, we feel that therapeutic approaches should be directed toward decreasing degenerative components of the s-IBM pathogenesis. Unfortunately, thus far the source of a Fountain of Youth has not yet been identified.

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